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733-6 High Rates of Apoptosis in Human Coronary Atherosclerotic Lesions Lacking Compensatory Enlargement

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Compensatory enlargement of atherosclerotic arteries is an adaptive process that is thought to determine vascular lumen size. Apoptosis of cells of the vascular wall may modulate the cellularity of lesions that produce vascular obstruction. To investigate the association of apoptosis with adaptive changes in vessel size triggered by atherosclerosis, intravascular ultrasound was performed in 23 severe primary coronary stenoses prior to directional coronary atherectomy. Compensatory enlargement (CE+) was defined as an increase in vessel cross-sectional area at the lesion site of at least 10% compared to the proximal reference site. Lesions failing to show compensatory vessel enlargement were classified as (CE−). Atherectomy specimens were analyzed utilizing in situ labeling of DNA strand breaks (TUNEL). Cells were classified as TUNEL-positive (TUNEL+) when both positive immunoreactivity and key morphologic features of apoptosis (e.g., pycnosis) were present. Apoptotic indices were obtained dividing the number of TUNEL+ cells by the total number of counted cells per field (Total).

Results:

Group	TUNEL+ (± SD)	Total (± SD)	Index (± SD)
CE+ (n = 9)	7.8 ± 5.2	240.7 ± 69.1	3.0 ± 1.9
CE− (n = 14)	21.4 ± 17.4	233.5 ± 121.4	11.2 ± 9.1
p-value	*0.035*	*0.87*	*0.015*

In conclusion, an increased number of TUNEL+ cells was associated with lesions lacking compensatory vessel enlargement. Identification of signals and mechanisms underlying increased apoptosis in (CE−) lesions will contribute to a better understanding of arterial remodeling.

734 Platelets and Coronary Thrombosis

Tuesday, March 18, 1997, 8:30 a.m.–10:00 a.m.
Anaheim Convention Center, Room C1

8:30

734-1 The Benefits of Brief Antecedent Ischemia on Platelet-Mediated Thrombosis are Mimicked by Brief Intracoronary Adenosine Infusion

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Patients with angina prior to myocardial infarction exhibit enhanced recovery of coronary perfusion following thrombolysis by an as-yet unknown mechanism. We propose that adenosine released during brief antecedent ischemia/reperfusion may attenuate platelet aggregation and thereby contribute to improved vessel patency. To test this theory, 25 dogs underwent arterial injury + coronary stenosis, resulting in repeated cyclic variations in coronary flow (CFV's) caused by the spontaneous formation/dislodgement of platelet thrombi. Immediately before injury + stenosis, 10 dogs received 10 min of antecedent, mechanically-induced coronary occlusion (CO) followed by 10 min of reflow, 5 received a 10 min intracoronary infusion of adenosine (400 μg/min) and 10 min of washout, while 10 served as time-matched controls. Coronary blood flow was monitored at baseline, during the 20 min intervention period, and for 1 h post-stenosis. Vessel patency was compared by quantifying the number of CFV's, the duration of total thrombotic occlusion (flow = 0), and the area of the flow-time profile (expressed as a % of baseline flow × 60 min):

	# CFV's	Zero flow	% Flow-time area
Control	3 ± 1	28 ± 8 min	20 ± 8%
Brief CO	7 ± 2	10 ± 6 min	45 ± 7%
Adenosine	18 ± 6	10 ± 5 min	55 ± 10%

Baseline and stenotic blood flows were comparable among the groups and, as expected, all dogs developed platelet aggregation/dislodgement following injury + stenosis. Controls exhibited ≅ 3 CFVs, the duration of total thrombotic occlusion was 28 min, and the flow-time area was 20% of baseline. In contrast, both brief antecedent CO and brief adenosine infusion were associated with a greater incidence of CFVs, a reduction in zero flow duration to 10 min, and an increase in the flow-time area to 45–55% (p = 0.01, 0.06 and 0.03 vs controls, respectively). Thus, the benefits of brief ischemia/reperfusion on platelet-mediated thrombosis in damaged and stenotic canine coronary arteries are mimicked by brief infusion of adenosine, implying that adenosine may contribute to the improved vessel patency seen with antecedent angina.

8:45

734-2 Low Intracellular Magnesium Levels Promote Platelet-Thrombus Formation In Patients With Coronary Artery Disease

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Although reduced intracellular levels of magnesium ($[Mg]_i$) have been described in patients with acute MI, its significance remains unknown. To determine whether reduced $[Mg]_i$ enhances platelet-dependent thrombosis (PDT), we measured PDT in 42 stable CAD patients on aspirin and lipid-lowering therapy by exposing porcine aortic media to their flowing venous blood for 5 min at a shear rate of 800 sec^{-1} at 37ºC using an ex-vivo chamber model. PDT was measured by computerized morphometry and expressed as $\mu m^2/\mu m^2$ of the aortic surface. The $[Mg]_i$ in isolated mononuclear cells was measured by atomic absorption spectrophotometry (normal range: 1.23 ± 0.02 μg/mg protein). Patients were divided into two groups: Group A below and Group B above the median $[Mg]_i$ level (1.11 μg/mg protein).

Results:

	$[Mg]_i$ (μg/mg protein)	PDT ($\mu m^2/\mu m^2$)
Group A (n = 21)	0.98 ± 0.09	3.10 ± 2.70
Group B (n = 21)	1.37 ± 0.28*	0.99 ± 0.63$^{\alpha}$

Values are mean ± SD; *p < 0.0001, $^{\alpha}$p < 0.03 vs Group A

There were no significant differences in age, serum total cholesterol, LDL-C, HDL-C, triglycerides, fibrinogen, or serum magnesium levels between the two groups. *Conclusion:* Platelet-dependent thrombosis is significantly increased in stable CAD patients with low $[Mg]_i$. This finding demonstrates that low intracellular magnesium levels may favor arterial thrombus formation in CAD patients, suggesting a potential role for magnesium supplementation in CAD.

9:00

734-3 Inducible Nitric Oxide Synthase is Associated With Thrombi After Experimental Angioplasty and in Advanced Human Coronary Atherosclerotic Plaques

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The purpose of this study was to localise inducible NO synthase (iNOS) expressing cells after balloon angioplasty of the rabbit carotid artery, and in human coronary atherosclerotic plaques that are the substrate for therapeutical angioplasty.

The carotid artery of New Zealand white rabbits was dilated with a 2.5 mm balloon and examined after 1, 2 and 3 weeks. The sections were stained with antibodies against smooth muscle cells, endothelial cells, fibrin(ogen), von Willebrand factor (vWF), platelets (CD31), macrophages (RAM-11 or CD68) and iNOS. After angioplasty RAM-11 and iNOS reactive cells were present in the adventitia and in intramural thrombi, but not in the media. The number of iNOS reactive cells was proportional to the number of RAM-11 reactive cells. Smooth muscle cells were not reactive for iNOS. In the thrombi the number of iNOS and RAM-11 reactive cells increased significantly the first 3 weeks, whereas their number did not change in the adventitia.

		1 week (n = 5)	2 weeks (n = 6)	3 weeks (n = 17)
Thrombus	iNOS	0.6 ± 0.6	1.7 ± 0.5	2.6 ± 0.2*
	RAM-11	1.8 ± 0.6	3.3 ± 0.5	3.8 ± 0.1*
Adventitia	iNOS	1.8 ± 0.5	2.0 ± 0.4	1.0 ± 0.2
	RAM-11	3.0 ± 0.3	2.9 ± 0.3	2.6 ± 0.2

Mean score ± SEM, *p ≤ 0.01 vs week 1.

In human coronary atherosclerotic plaques, obtained by atherectomy, iNOS was present in macrophages of ruptured plaques and the associated thrombi, and in macrophages around the necrotic core.

Conclusion: In the rabbit angioplasty lesions, the iNOS immunoreactivity was present in macrophages, that were mainly found in colonising intramural thrombi and the adventitia. In non-ruptured human coronary plaques, iNOS was also found in macrophages around the necrotic core and the calcifications. The ruptured atherosclerotic plaques showed additionally a dense infiltration of the fibrous cap by iNOS expressing macrophages.